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Biokinetic modeling of Cd bioaccumulation from water, diet and sediment in a marine benthic goby: a triple stable isotope tracing technique

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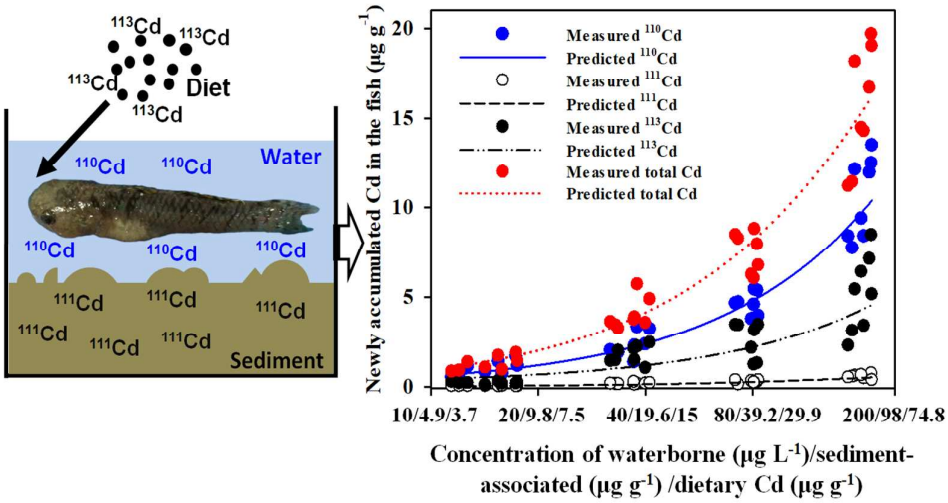
Abstract

Aquatic animals are often simultaneously exposed to metals through multiple routes in the natural environment. This study explored a triple stable isotope tracing method to quantify simultaneous cadmium (Cd) uptake biokinetics by yellow stripe goby from water (traced by ^{110}Cd), sediment (traced by ^{111}Cd), and diet (traced by ^{113}Cd) when the fish were exposed to Cd for 24 h. The simultaneous uptake of Cd from multiple routes during 4 weeks was then predicted by the modified biokinetic model. The results demonstrated that the uptake rate constant of waterborne ^{110}Cd, sediment-associated ^{111}Cd, and dietary ^{113}Cd was 3.1 L kg^{-1} d^{-1}, 2.2×10^{-4} g g^{-1} d^{-1} and 3.3×10^{-3} g g^{-1} d^{-1} in the fish. Sedimentary Cd was less bioavailable than the waterborne and dietary Cd, however, sediment could become the predominant Cd source of the total Cd bioaccumulation when the partition coefficient of Cd between sediment and seawater (K_d) is larger than 6×10^4 L kg^{-1}. The simultaneous uptake of Cd from the three routes could be successfully predicted by the modified model. The model revealed that the Cd bioaccumulation generally increased with the increase of ambient Cd concentration in all the three routes. Overall, our findings demonstrated that the multiple stable isotopes tracing method and the modified biokinetic model have a wide generality and applicability for predicting Cd bioaccumulation under multiple routes of metal exposure scenario and may have application to other metals.

Key words: biokinetics, cadmium, fish, sediment, multiple routes of exposure

Capsule*: The simultaneous uptake of Cd from water, sediment and diet is successfully predicted by the modified biokinetic model in a benthic fish.*

TOC/Abstract Graphic



Highlights

1) The simultaneous uptake of Cd from multiple routes was predicted by the modified biokinetic model

2) The sediment-associated Cd was of low bioavailability to the benthic goby at the present exposure condition

3) Multiple stable isotope tracing method is useful for studying multiple routes of metal uptake

1. Introduction

The wide metal contamination in aquatic environments has been a global concern over the past decades, prompting an increasing need to understand the toxicity and bioaccumulation of elevated metals in aquatic organisms. In the environment, aquatic animals are often simultaneously exposed to multiple routes of elevated metals. Historically, studies of metal bioaccumulation mainly focused on waterborne metal uptake with emphasis on the chemistry of metals in aquatic environments.[1,2] However, dietary exposure is an important route of metal bioaccumulation in many aquatic animals.[3-8] The biokinetic model was developed to quantitatively investigate the key processes determining metal bioaccumulation in aquatic organisms.[6,7] Most previous studies deploying the biokinetic model examined metal bioaccumulation from the waterborne and/or dietary routes in separate experiments.[6,8] For instance, many studies used fasted aquatic animals (e.g., starved 24 h) when they determined the waterborne metal uptake.[8] Similarly, dietary metal uptake was typically measured in absence of waterborne exposure.[3-5] Thus the present state of knowledge deriving from the separate single route of metal exposure is incomplete in informing the picture of the simultaneous uptake of metals from multiple routes as it occurs in the environment.[9,10]

The interaction between the waterborne and dietary metal uptake has been demonstrated in aquatic animals. For example, long-term pre-exposure to waterborne nickel downregulated gastrointestinal nickel uptake in rainbow trout (*Oncorhynchus mykiss*)[11]. Rainbow trout fed a Cu deficient diet up-regulated branchial Cu uptake, but down-regulated branchial Cu uptake when fed a Cu supplemented diet.[12] Our previous studies indicated that the Cu uptake from water route could be substantially affected by the Cu from the dietary route in juvenile rabbitfish (*Siganus oramin*).[9] We further demonstrated a significant influence of waterborne Cd exposure on the uptake of dietary Cd at given exposure condition in marine yellowstripe goby.[10] To our knowledge, however, there has been little effort made to understand and validate metal uptake using the biokinetic model in aquatic organisms when they simultaneously take up metal from multiple routes since most previous studies with

the model used separated single exposures, largely owing to the lack of a multiple stable isotope tracing technique.

In aquatic ecosystem, metal contaminated sediments have been widely known as an important sink for metals and metalloids, playing a significant role in the transport and storage of metals, especially in aquatic benthic habitats.[13,14] Attempts have been made to investigate the bioavailability and toxicity of sediment-associated metals to benthic fauna, mostly on polychaetes and bivalves.[16-18] To date, there has been surprisingly scarce information on sediment-associated metal bioaccumulation in benthic fish species, despite the fact that many benthic fish are sediment-dwelling/deposit feeding and have burrowing behavior for feeding and/or breeding.[19] Therefore, there is a need to quantitatively characterize the sediment-associated metal uptake in benthic fish.

In this study, therefore, we set out to explore a triple stable isotope tracing method (^{110}Cd, ^{111}Cd and ^{113}Cd) to quantify the Cd uptake biokinetics from water, sediment and diet simultaneously in the marine yellowstripe goby (*Mugilogobius chulae*).[10,20] The general aim here was to model simultaneous uptake of this metal *via* multiple routes in benthic marine fish. Within the framework of the biokinetic model,[6,7] we first quantified Cd uptake kinetics by a short-term Cd uptake experiment (24 h),[10] and then we determined the Cd efflux of the fish in a 4-week depuration experiment. Finally, we proposed a modified biokinetic model to predict the Cd bioaccumulation from water, diet, and sediment in the fish, and the prediction was then validated by a 4-week Cd exposure experiment.

2. Materials and methods

2.1. Experimental fish and metals

The marine yellowstripe goby (*Mugilogobius chulae*, 1.1±0.14 g) were obtained from Guangdong Laboratory Animals Monitoring Institute (Guangzhou, P. R. China). In the laboratory aquaria, the fish were acclimated 2 weeks, during which they were fed clam (*Ruditapes philippinarum*). The shell of the clam was removed, and then the clam was oven-dried and crushed to chips with diameters less than 2 mm. The initial

Cd content in the clam was 0.18 ± 0.07 μg g^{-1} in dry matter.

The stable isotope ^{110}Cd (purity of 96%), ^{111}Cd (purity of 97%) and ^{113}Cd (purity of 93%) all as the crystal and monomer were purchased from International Atomic Energy Agency Office at USA, New York), and were used as tracers to quantify Cd uptake from water, sediment and diet, respectively. The other Cd source used was $CdCl_2$ (Sigma-Aldrich), which contained Cd with natural isotopic ratios.

2.2. Sediment preparation, Cd equilibration in the water and sediment

The sediment used in the study was sampled from Daya Bay, Guangdong Province, South China (114°40′ E, 22°40′ N). The surface sediment was collected and sieved in the laboratory (mesh size of 0.43 mm). It was washed using distilled water for 5 times and then washed using seawater 3 times to re-establish the salinity. The Cd content in the sediment was 0.025 ± 0.0031 μg g^{-1} DW with natural isotope contents (n= 4).

The preliminary experiment was conducted to determine the Cd equilibration in the Cd spiked sea water and sediments (STable 1). Specifically, the seawater was first spiked with Cd with naturally occurring isotopic ratios up to 20 (named T1), 50 (named T2), 100 (named T3), and 200 μg L^{-1} Cd (named T4), respectively (0.19, 0.45, 0.89, and 1.8 μM L^{-1}). Then the prepared sediment and spiked sea water (1:3 g ml^{-1}) were mixed thoroughly in 18-L aquaria, homogenized for 15 minutes and shaked for 30 minutes. Then, the equilibration of Cd in the sea water and sediment was investigated for 4 weeks, during which the samples were taken each week. The Cd content in the sea water and sediment was relatively stable in the 4^{th} week, suggesting the completion of the Cd equilibration (STable 1). Thus, the Cd concentration of water and sediment at the end of the equilibration was the reference for the following Cd uptake experiments (STable 1).

2.3. Stable isotope Cd spiking in water, sediment and diet

The seawater was filtered using Whatman glass filters (pore size 0.22 mm) followed by addition of the solution containing ^{110}Cd to obtain the required concentration of

15, 40, 87, and 178 μg L^{-1} in the T1-T4 (0.14, 0.36, 0.78, and 1.6 μM L^{-1}), according to the results in the preliminary experiment (STable 1).

The nominal Cd content in the sediment was set as 6, 22, 45, and 90 μg g^{-1} in T1-T4, respectively (0.054, 0.20, 0.41, and 0.82 μM g^{-1}, SFigure 1&2). ^{111}Cd was used as the tracer. Specifically, a solution containing a known volume of ^{111}Cd was added into the prepared sediment for each concentration (1:3 g ml^{-1}). The sediment was then homogenized for 15 minutes and shook for 30 minutes.

The fish diets (i.e. clam) were spiked with ^{113}Cd. The clam was exposed in seawater containing ^{113}Cd at concentrations of 15, 40, 87, and 178 μg L^{-1} for 4 weeks. At end of the exposure, the measured ^{113}Cd content in the T1-T4 clam was 3.5 ± 0.77, 21 ± 3.08, 34 ± 6.5, 60 ± 7.2 μg g^{-1}, respectively (DW, n = 3).

2.4. Short-term Cd uptake experiment

The short-term Cd uptake experiment was conducted in beakers for 24 hours (h). The ^{110}Cd spiked sediment and ^{111}Cd spiked seawater was added to the beakers, which were then equilibrated for 48 h before Cd uptake experiment (the initial sediment and seawater samples were taken after the equilibration).

Two hundred fish were individually fed in beakers (1 fish per beaker) for 1 week to acclimate to the experiment conditions, during which the fish were not exposed to Cd. The fish were starved for 48 h, eight fish were sampled and then fed with ^{113}Cd spiked diets for 1 h. After feeding, the fish were individually transferred to the prepared beaker that contained ^{110}Cd spiked sediment and ^{111}Cd spiked seawater. The uptake experiment was 24 h as the chyme evacuation time was 24 h and the fish finished the dietary Cd uptake within 24 h based on our previous study.[10] Fish were killed by an overdose of MS-222 at 0, 2, 4, 8, 12, 18 and 24 h. At each sampling time point, 8 fish were sampled and 192 fish were sampled totally in the T1-T4. The Cd concentrations in the sediment and seawater were measured at 0, 6, 12, 18, and 24 h. The variation of Cd concentration was less than 9%.

2.5. Cd efflux experiment

Sixty-four fish were first exposed to waterborne ^{113}Cd (178 μg L^{-1}) and dietary ^{113}Cd (69 μg g^{-1}) for two weeks to accumulate ^{113}Cd. They were then maintained in clean seawater for a four-week depuration period, and the fish were sampled at 0, 2, 6, 10, 14, 18, 22, and 28 d (8 fish were sampled each time). The fish were fed twice a day and the seawater was renewed daily. The ^{113}Cd concentration in seawater was measured at 0, 2, 6, 10, 14, 18, 22, and 28 d during the depuration. There was no significant change in the ^{113}Cd concentration during the depuration period (0.68 ± 0.08 μg L^{-1}).

2.6. Long-term Cd uptake experiment

The long-term Cd uptake experiment (28 days (d)) was conducted in 18 L aquaria. The ^{111}Cd spiked sediment was placed in the aquaria, and the ^{110}Cd spiked seawater was then carefully added. The aquaria were then equilibrated for 48 h (the initial sediment and seawater samples were taken after the equilibration).

The juvenile marine yellowstripe goby was fed in the aquaria for one week to acclimate to the experiment condition without Cd exposure. The fish were starved for 48 h and then transferred to the prepared aquaria that contained ^{111}Cd spiked sediment and ^{110}Cd spiked seawater.

During the long-term Cd uptake experiment, the fish were fed with the ^{113}Cd spiked diets once a day. The feeding ration was 15% of the fish body weight, similar to the natural feeding rate in this fish species. Fish feces were siphoned off gently each day. The fish, sediment, overlaying water (the water sampled from 2-3 cm above the sediment) and seawater (the water sampled from 0.5-1 cm below the surface of the water) were carefully sampled at 0, 7, 14, 21 and 28 d (8 fish were sampled and 128 fish were sampled totally in the T1-T4.). The pore water in the sediment was extracted by centrifuging of the sediment at 3500 rpm for 10 min. The Cd concentrations in fish kept constant as 0.21 ± 0.09 μg g-1 in the control treatment.

2.7. Stable isotope Cd concentration analysis

Whole fish were oven-dried, ground to powder, digested in 1 ml of HNO_3 for 48 h at 80 °C. The sediment was oven-dried at 80 °C for 48 h, ground and sieved. Then, about 0.2 g sediment was digested with 2 ml HNO_3 for 48 h at 80 °C, and the digested samples were diluted and filtered. The marine sediment reference material for trace metals (PACS-3, National Research Council Canada) and blank samples were analyzed with the same procedures as the check samples. The recovery rate of Cd was 91-96%. ^{115}In was selected as internal standard to correct the sensitivity drift and matrix effect during the analysis. A quality control sample was repeatedly measured every 20 samples. Then the content of total Cd and stable isotope (^{110}Cd, ^{111}Cd and ^{113}Cd) in the digested samples were quantified by inductively coupled plasma-mass spectroscopy (ICP-MS, 7700X, Agilent Technologies Inc., California, USA). The net change of ^{110}Cd, ^{111}Cd and ^{113}Cd in the samples were calculated as described by Guo et al.[10] and Croteau et al.[18] (see detailed equation in Supporting information).

2.8. Biokinetic modeling and statistical analysis

The biokinetic model was applied to analyze and simulate Cd bioaccumulation in the fish. The model is mechanistically based, considering the metal bioaccumulation as a gross result of metal uptake from different routes and elimination (including growth dilution). Biokinetic modelling has succeeded in explaining and predicting total bioaccumulated concentrations of different metals in many aquatic organisms.[6,7] In the present study, the biokinetic model was modified to describe the Cd bioaccumulation from water, sediment and diet as defined:

$$dC_t / dt = k_{\text{u-wat}} \times C_{\text{w}} + k_{\text{u-sed}} \times C_{\text{s}} + k_{\text{u-diet}} \times C_{\text{f}} - (k_{\text{e}} + g) \times C_t \quad (1)$$

where C_t is the Cd concentration in the fish at time t, $k_{\text{u-wat}}$ is the uptake rate constant of waterborne Cd (L g^{-1} d^{-1}), C_{w} is the waterborne Cd concentration (μg L^{-1}), $k_{\text{u-sed}}$ is the uptake rate constant of sediment-associated Cd (g g^{-1} d^{-1}), C_{s} is the sediment-associated Cd concentration (μg g^{-1}), $k_{\text{u-diet}}$ uptake rate constant of dietary Cd (g g^{-1} d^{-1}), C_{f} is the Cd concentration in diet (μg g^{-1}), k_{e} is the Cd efflux rate constant (d^{-1}), and g is the fish growth rate (d^{-1}).

The waterborne ^{110}Cd uptake rate ($J_{\text{in-wat}}$, μg g^{-1} d^{-1}) was first calculated as the slope of the linear regression between the newly bioaccumulated ^{110}Cd (the bioaccumulation of ^{110}Cd from the sediment was ignored as the estimated contribution to total ^{110}Cd bioaccumulation was less than 1%) and exposure time. The $k_{\text{u-wat}}$ was then determined as defined:

$$k_{\text{u-wat}} = J_{\text{in-wat}} / C_{\text{w}}^{\text{b}} \quad (2)$$

where b is the is the kinetic order.

The sediment-derived ^{111}Cd uptake rate ($J_{\text{in-sed}}$, μg g^{-1} d^{-1}) was calculated as:

$$J_{\text{in-sed}} = (\text{total } ^{111}\text{Cd concentration} - J_{\text{in-wat}} \times {}^{111}C_{\text{w}} \times \text{exposure time}) / \text{exposure time} \quad (3)$$

where $^{111}C_{\text{w}}$ was the $^{111}C_{\text{w}}$ concentration in the water.

The $k_{\text{u-sed}}$ of ^{111}Cd was thus determined as defined:

$$k_{\text{u-sed}} = J_{\text{in-sed}} / C_{\text{s}}^{\text{b}} \quad (4)$$

where b is the is the kinetic order.

The $k_{\text{u-diet}}$ of diet-derived ^{113}Cd was then determined as defined:

$$k_{\text{u-diet}} = \text{AE} \times \text{IR} \quad (5)$$

where the AE is the diet-derived Cd assimilation efficiency. $\text{AE} = \text{A}_{24\,\text{h}} / \text{A}_{0\,\text{h}}$, where $\text{A}_{24\,\text{h}}$ was the calibrated dietary ^{113}Cd contents retained in fish at 24 h and it was calculated as:

$$\text{A}_{24\,\text{h}} = \text{total } ^{113}\text{Cd concentration} - J_{\text{in-wat}} \times {}^{113}C_{\text{w}} \times \text{exposure time} \quad (6)$$

$\text{A}_{0\,\text{h}}$ is the initial ^{113}Cd in fish after diet ingestion. IR is the fish diet ingestion rate defined as：

$$\text{IR} = (\text{the fed diet} - \text{the collected uneaten diet}) / \text{fish body weigh t/days} \quad (7)$$

The k_{e} was calculated from the slope of the linear regression between the natural logarithm of the percentage of ^{113}Cd and depuration time.

The fish specific growth rate (g) was calculated as (STable 2):

$$g = \ln(\text{fish final body weigh t} / \text{fish initial body weigh t}) / \text{days} \quad (8)$$

The relative importance of ^{110}Cd accumulated from the water (f_w, %), ^{111}Cd accumulated from the sediment (f_s, %), and ^{113}Cd accumulated from the diet (f_d, %) was calculated as:

$$f_w = k_{u\text{-}wat} / (k_{u\text{-}wat} + k_{u\text{-}sed} \times K_d + k_{u\text{-}diet} \times \mathrm{BCF}) \times 100 \quad (9)$$

$$f_s = k_{u\text{-}sed} \times K_d / (k_{u\text{-}sed} \times K_d + k_{u\text{-}wat} + k_{u\text{-}diet} \times \mathrm{BCF}) \times 100 \quad (10)$$

$$f_d = 100 - f_w - f_s \quad (11)$$

where BCF (L kg^{-1}) was the bioconcentration factor of Cd in diets and K_d (L kg^{-1}) was the partition coefficient of Cd between sediment and seawater, which was calculated as:

$$\mathrm{BCF} = C_f / C_w \quad (12)$$

$$K_d = C_s / C_w \quad (13)$$

In the determination of the Cd bioaccumulation in the long-term experiment, the calibration was also conducted. Specifically, the concentrations of ^{110}Cd originating from waterborne exposure in the fish were not adjusted because the bioaccumulation of ^{110}Cd from the sediment was ignored since the estimated contribution to total ^{110}Cd bioaccumulation was less than 1%. The concentrations of the sediment-associated ^{111}Cd in the fish (C_{sed}) were calibrated as:

$$C_{sed} = (\text{total } ^{111}\text{Cd concentration} - J_{in\text{-}wat} \times {}^{111}C_w \times \text{exposure time}) \quad (14)$$

The concentrations of the dietary ^{113}Cd in the fish (C_{diet}) were calibrated as:

$$C_{diet} = \text{total } ^{113}\text{Cd concentration} - J_{in\text{-}wat} \times {}^{113}C_w \times \text{exposure time} \quad (15)$$

All statistical analyses were performed by the SPSS software package (vs. 18, SPSS Inc., Chicago, USA).

3. Results

3.1. The verification of Cd contents in the sediment and water

The concentrations of waterborne ^{110}Cd, ^{111}Cd, and ^{113}Cd in the water and overlying water were almost the same and stable during the 28-d experiment (SFigure 1).

Therefore, it was not necessary to separate the normal water and overlying water layers in this study. Although the sediment-associated ^{111}Cd and dietary ^{113}Cd were detected in the water, they took a very small proportion of the total Cd (<5% for ^{111}Cd or ^{113}Cd). Therefore, ^{110}Cd could well trace the waterborne Cd uptake in this study.

The concentrations of Cd isotopes in the sediment were also stable during the experimental period (SFigure 2). ^{111}Cd was always the predominant Cd isotope (>90%) in the sediment, suggesting that ^{111}Cd was also a good tracer for sedimentary Cd uptake in this study. Similarly, ^{113}Cd was the predominant Cd isotope (>99%) in the clam and traced well the dietary Cd uptake.

3.2. The Cd uptake and efflux kinetics

The waterborne ^{110}Cd uptake rate (J_{in}) was 31±1.3, 74±3.2, 129±8.9, 240±9.4 ng g^{-1} d^{-1} in T1-T4, respectively (Figure 1A). The uptake rate constant of waterborne ^{110}Cd ($k_{u\text{-}wat}$) was calculated as 3.1±0.14 L kg^{-1} d^{-1} (Figure 1C).

The J_{in} of sediment-associated ^{111}Cd was 1.45±0.055, 6.0±0.26, 11±0.53, and 23±1.3 ng g^{-1} d^{-1} in the T1-T4 (Figure 1B), and the calculated ^{111}Cd $k_{u\text{-}sed}$ was 2.2±0.11 × 10^{-4} g g^{-1} d^{-1} (Figure 1D).

The dietary ^{113}Cd assimilation efficiency (AE) was 1.76-2.45 % (Figure 1E), and the calculated $k_{u\text{-}diet}$ was 3.0±0.093, 3.3±0.13, 3.4±0.19 and 3.6±0.25 × 10^{-3} g g^{-1} d^{-1} in the T1-T4 with the mean of 3.3±0.17 × 10^{-3} g g^{-1} d^{-1}.

The Cd efflux rate constant (k_e) was 0.041±0.0022 d^{-1} (Figure 1F).

3.3. Cd bioaccumulation during 28-d Cd exposure

The waterborne ^{110}Cd content in the fish increased steadily with the exposure time (Figure 2A). The sediment-associated ^{111}Cd concentration in the fish was 0.07, 0.21, 0.33, 0.68 μg g^{-1}, which was about 11 to16-fold lower than ^{110}Cd (Figure 2B). Dietary ^{113}Cd contents in the fish was only slightly lower than the values of the waterborne ^{110}Cd (Figure 2C). Totally, the Cd concentration was up to 1.5, 4.3, 8.2, and 17 μg g^{-1} in the T1-T4 at the end of 28-d triple routes of Cd exposure (Figure

2D).

3.4. The modeling of Cd bioaccumulation from water, sediment and diet

Other than the biokinetics above, the parameters used for the modeling included the ingestion rate (IR, 0.14, 0.14, 0.140 and 0.15 g g^{-1} d^{-1} in the T1-T4, respectively) and specific growth rate (g, 0.0093, 0.0094, 0.0095 and 0.0092 d^{-1} in the T1-T4, respectively). The modified biokinetic model successfully estimated the waterborne (Figure 3A), sediment-associated (Figure 3B), and dietary Cd (Figure 3C) bioaccumulation in the fish simultaneously exposed to the three sources of Cd for 28 days. Moreover, the total Cd bioaccumulation also could be estimated well (Figure 3D).

Generally, the prediction revealed that the Cd bioaccumulation increased steadily with the increase of ambient Cd concentration through the three routes of Cd exposure. At the present K_d of 1.8×10^3-2.7×10^3 L kg^{-1} and BCF of 2.5-4.1×10^3 L kg^{-1}, the relative importance of waterborne ^{110}Cd (f_w, %) ranged from 42.1 to 70.4 % (Figure 4A), and that of sediment-associated ^{111}Cd (f_s, %) ranged from 7.6 to 15.8 % (Figure 4B), both of which showed a slightly decrease with the increase in ambient Cd concentration. However, the relative importance of dietary ^{113}Cd (f_d, %) ranged from 19 to 51 %, and it increased from 37 % to 69 % as the ambient Cd level increased from 15 to 40 μg L^{-1}(Figure 4C).

Moreover, the modeling further indicated that the f_s exponentially increased with an increase of K_d at a given BCF, while the f_s deceased when the BCF increased (Figure 5A). Similarly, the f_d exponentially increased with the increase of BCF at a given K_d, and it deceased when the K_d increased (Figure 5B).

4. Discussion

4.1. The modeling of simultaneous uptake of Cd from three routes in the benthic fish

Although the biokinetic model has been in development for more than 30 years, our study was the first to demonstrate that the simultaneous uptake of metal from three different routes can be successfully predicted by the modified model in the aquatic

394 animals, apparently differing from previous studies using a separate single metal
395 exposure in the model.[6,7] Within the range of Cd concentrations used in the present
396 study (i.e., waterborne Cd of 15-200 μg L^{-1}, sediment-associated Cd of 6-90×10^{-3} μg
397 kg^{-1}, and dietary Cd of 4-60×10^{-3} μg kg^{-1}), the model was accurate in predicting the
398 Cd bioaccumulation from the multiple routes of water, sediment, and diet when the
399 fish were simultaneously exposed to Cd *via* three routes. Our previous findings
400 revealed that the uptake of a single route of metals sometimes can be significantly
401 affected by another route of metal exposure.[9,10] This was in line with the Cd uptake
402 biokinetics observed in this experiment in relation to our previous results under
403 single exposure. Specifically, the AE of dietary ^{113}Cd (1.76-2.45 %) under multiple
404 routes of Cd exposure was lower than 3.24 % under single dietary Cd exposure.[10]
405 The $k_{u\text{-}wat}$ of ^{110}Cd in this study (3.1 L kg^{-1} d^{-1}) was also slightly lower than that
406 under single waterborne Cd exposure (3.48 L kg^{-1} d^{-1}).[10] The parameters (AE and
407 $k_{u\text{-}wat}$) of the biokinetic modeling were overestimated by detected under single route
408 of exposure than simultaneous multiple exposure. Therefore, the biokinetic modeling
409 still showed a greater promise for interpreting and predicting the metal accumulation
410 in fish from simultaneous multiple exposure pathways.

411 Moreover, it is noteworthy that the triple stable isotope tracer method (i.e.,
412 ^{110}Cd, ^{111}Cd and ^{113}Cd) explored in the present study was a novel and useful method
413 that successfully facilitated the spiking of the Cd derived from different routes. More
414 recently, Tang et al. have also shown that the a dual Cd stable isotope method was
415 able to greatly facilitate the determination of size-dependent metal accumulation
416 from different routes in zebra mussel (*Dreissena polymorpha*).[20] Therefore, we
417 propose that the multiple stable isotope tracer method is a highly applicable for
418 studies aimed to determine/predict the simultaneous uptake of metal from multiple
419 routes and/or the interaction of metal uptake between different routes of metals,
420 which has been largely overlooked previously but increasingly highlighted, given the
421 fact that aquatic organisms are often simultaneously exposed to multiple routes of
422 metals in the environment.[9,10,21]
423

4.2. The uptake and relative importance of Cd bioaccumulation from three routes

Most models to predict metal bioaccumulation in fish comes from experiments with exposure *via* the waterborne or dietary phase, while there is nearly no available information on the bioavailability of sediment-associated metals, even though metals have long been known to be in high concentrations in sediments.[22,23] This study was an initial attempt to determine the sediment-associated metal uptake biokinetics in a fish species. Previous studies demonstrated that the predominantly sediment dwelling taxa took up the majority of their Cd from the water compartment (*via* overlying water, porewater and/or burrow water),[24-26] and only sediment feeding midges and worms took up substantial amounts of Cd from the sediment compartment.[24,25] The dominant metal exposure route in the typical deposit-feeders and/or suspension-feeders (e.g., amphipods and bivalves) is through the ingestion of metal-contaminated particles.[16,21,25,27] For benthic fish, however, there has been little information to date regarding this issue. Therefore, further efforts are highly needed to quantitatively determine the uptake and relative importance of sediment metal *via* dissolved route (overlying waterborne Cd, and/or pore waterborne Cd) and particle-associated route in fish.[28]

In the triple route exposure condition assessed in the present study, the relative importance of sediment-derived Cd in the fish was 7-15%. Consistently, the $k_{u\text{-}sed}$ was 2.2×10^{-4} g g^{-1} d^{-1}, which was much lower than that of waterborne Cd (3.1±0.14 ml g^{-1} d^{-1}) and dietary Cd (3.3×10^{-3} g g^{-1} d^{-1}, Figure 1), suggesting lower bioavailability of sediment Cd in the fish in relation to the waterborne and dietary routes. A small contribution of sediment-associated metal to the total amount of metal has been also observed in several previous studies. In zebra mussel (*D. polymorpha*), for instance, the sediment-Cd contribution was only 5-8% regardless of a big variation in body size.[21] Similarly, the sediment compartment of Cd was found to account for 9.8% of the total Cd bioaccumulation in oligochaete *T. tubifex*.[29] The low bioavailability of sediment-associated Cu has also been evidenced in oligochaete (e.g., *Lumbriculus variegatus*).[31,32]

In the present study, the relative contribution of waterborne Cd was 6~9-fold

higher than that of sediment-derived Cd. The distribution of the metals between the water and sediment phase, quantitatively described by partition coefficient (K_d, L kg^{-1}), is the key factor determining the relative importance of sediment-associated and waterborne metal .[22,28] Generally, the contribution of sediment-derived Cd ought to increase with the increase of K_d (Figure 5).[29] The K_d in the present exposure condition was 1.8×10^3-2.7×10^3 L kg^{-1}, which was low in relation to 10^3-5×10^5 for marine and estuarine sediments in field situations.[30] Therefore, the low K_d was one of main reasons for the relatively low contribution of sediment-derived Cd in the fish (Figure 5A). In the nature environments, there is a long-time deposition of Cd into the sediment, which usually leads to a higher K_d and higher Cd concentration in the sediment in relation to this study at the comparable levels of waterborne Cd exposure.[33] Therefore, the results of the present exposure condition might underestimate the importance of sediment-associated Cd in the benthic fish in comparison with field situations (Figure 5A). According to our modeling, sediment could become the predominant Cd source of the total Cd bioaccumulation (>50%) when K_d is larger than 6×10^4 L kg^{-1} (Figure 5A). It should be noticed that Cd $k_{u\text{-}sed}$ was constant for the sediment with Cd K_d as 10^3-5×10^5 in our modeling. However, in the natural condition, Cd $k_{u\text{-}sed}$ could decrease for the sediment with high K_d since sediment with high K_d will become mineralized over time and have lower bioavailability. So, the actual contribution of sediment to the overall Cd bioaccumulation should be lower than our modeling when K_d is high. Even so, our modeling revealed that sediment could become important Cd sources to the benthic fish.

In this study, the diet of the fish (clams) only accumulated ^{113}Cd *via* water rather than *via* both waterborne and dietary pathway, which was likely to lead to the lower Cd concentration in the experimental fish diet. In addition, the AE of Cd observed here was also lower than the values in our previous study in the same species (e.g. 3.48 %).[10] Therefore, the relative importance of dietary ^{113}Cd (f_d) was probably underestimated in the condition of our study. Our modeling indicated that the f_d was much higher than the values in the present study when the Cd contents in

the diet was increased with the increase of BCF (Figure 5B). A substantial field investigation would be needed to quantify the magnitude of the underestimation since there seems little consensus in the previous studies regarding the relative importance of metal bioaccumulation from sediment, water or diet compartment among different species.[29,32,34]

In summary, the present study investigated a scenario of simultaneous uptake ofCd from seawater, sediment and diet in the benthic marine yellowstripe goby, which could be successfully predicted by a modified biokinetic model. We furthermore revealed a low bioavailability of sediment-associated Cd to this benthic fish in absence of burrowing behavior and/or ingestion of sediment in the exposure condition of our experiments. The results from our study proposed that the triple stable isotope tracer method is a highly useful technique for determining/predicting the simultaneous uptake of metal from multiple routes and/or the interaction of metal uptake between different routes in aquatic animals. The prediction of metal bioaccumulation from multiple routes as validated in the present study has a wide generality and applicability for most aquatic animals that are in the environmentally exposed to metals via water, diet and sediment.

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611 **Figure Legends**

612

613 **Figure 1.** The newly accumulated waterborne ^{110}Cd (panel A) and
614 sediment-associated ^{111}Cd concentrations (panel B), and the regression of the uptake
615 rate of ^{110}Cd (panel C) and ^{111}Cd (panel D) with the ambient Cd concentration, and
616 the retention of ingested dietary ^{113}Cd (%) in the fish after the pulse feeding (panel
617 E), and the retention of the fish body ^{113}Cd burden after 2 weeks ^{113}Cd exposure
618 (panel F) in the yellowstripe goby. Values are means ± standard deviations (n = 8) in
619 each of the 4 treatments (T1-T4).

620

621 **Figure 2.** The measured time-course ^{110}Cd (panel A), ^{111}Cd (panel B), ^{113}Cd (panel C)
622 and total Cd (panel D) bioaccumulation (μg g^{-1}) in the yellowstripe gobyafterthe
623 28-d simultaneous multiple routes of Cd exposure. Values are mean ± standard
624 deviation (n = 8).

625

626 **Figure 3.** The model-predicted ^{110}Cd (panel A), ^{111}Cd (panel B), ^{113}Cd (panel C), and
627 total Cd (panel D) bioaccumulation from water, sediment and diet in the yellowstripe
628 goby exposed to multiple routes of Cd simultaneously. The solid lines are model
629 prediction and the points are measured values. The dotted lines are the 1:1 lines, and
630 r^2 is the coefficient of dependence of the 1:1 line between the predicted and
631 measured values (n = 32). The same horizontal axis scaling of waterborne ^{110}Cd was
632 used for the visual comparison of Cd bioaccumulation among different routes. The
633 concentrations of sediment-associated ^{111}Cd were 6, 22, 45, and 90 μg g^{-1} (panel B),
634 and that of dietary ^{113}Cd were 4, 21, 34 and 60 μg g^{-1} (panel C).

635

636 **Figure 4.** The model-predicted relative importance of waterborne ^{110}Cd (f_w, panel A),
637 sediment-associated ^{111}Cd (f_s, panel B), and dietary ^{113}Cd (f_d, panel C) accounting for
638 the total Cd in the yellowstripe goby after 28-d simultaneous multiple routes of Cd
639 exposure. The solid lines are model prediction and the points are measured values.
640 The dashed lines are the 1:1 lines, and r^2 is the coefficient of dependence of the 1:1

line between the predicted and measured values (n = 32). The same horizontal axis scaling of waterborne ^{110}Cd was used for the visual comparison of Cd bioaccumulation among different routes. The concentrations of sediment-associated ^{111}Cd were 6, 22, 45, and 90 μg g^{-1} (panel B), and that of dietary ^{113}Cd were 4, 21, 34 and 60 μg g^{-1} (panel C).

Figure 5. The model-predicted changes of relative importance of sediment-associated ^{111}Cd (f_s) and dietary ^{113}Cd (f_d) accounting for the total Cd with the increase of K_d (the partition coefficient between sediment and seawater) and BCF (the bioconcentration factor of Cd in diets) in the yellowstripe goby under simultaneous multiple routes of Cd exposure. In the modeling, the K_d ranged 10^3 to 5 $\times 10^5$ L kg^{-1} and BCF ranged 10^3 to 10^4 L kg^{-1}[1,3].

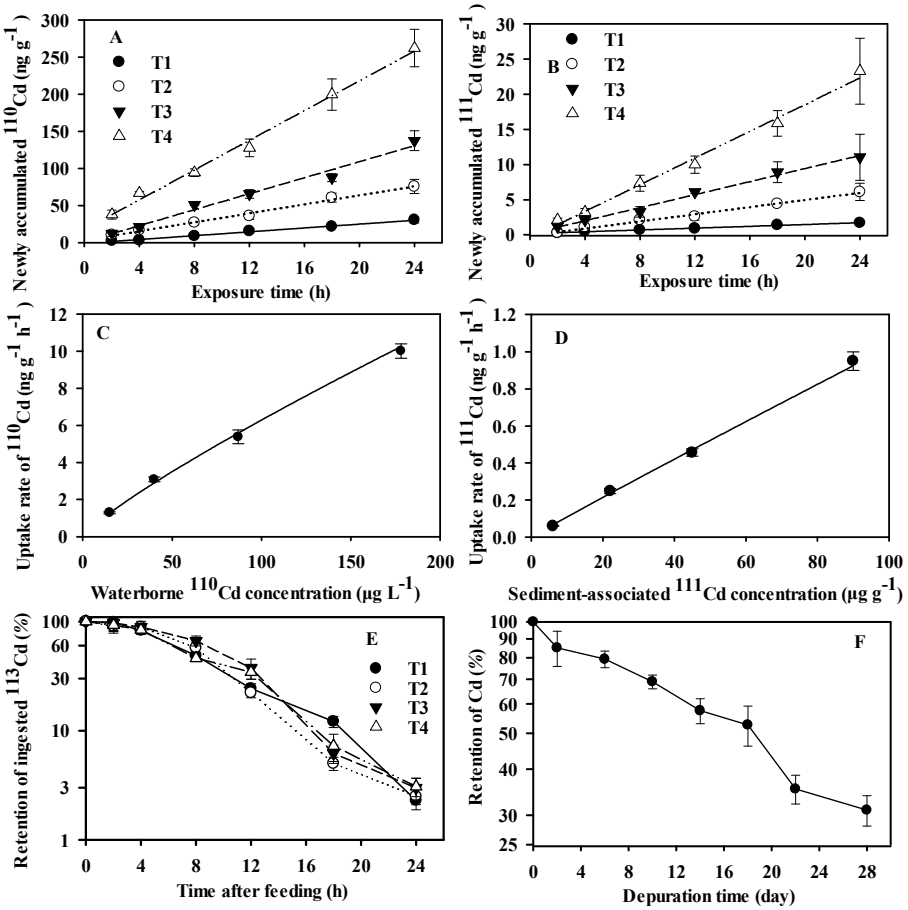

A
T1
T2
T3
T4
Newly accumulated ^{110}Cd (ng g^{-1})
Exposure time (h)
B
Newly accumulated ^{111}Cd (ng g^{-1})
C
Uptake rate of ^{110}Cd (ng g^{-1} h^{-1})
Waterborne ^{110}Cd concentration (μg L^{-1})
D
Uptake rate of ^{111}Cd (ng g^{-1} h^{-1})
Sediment-associated ^{111}Cd concentration (μg g^{-1})
E
Retention of ingested ^{113}Cd (%)
Time after feeding (h)
F
Retention of Cd (%)
Depuration time (day)

Figure 1

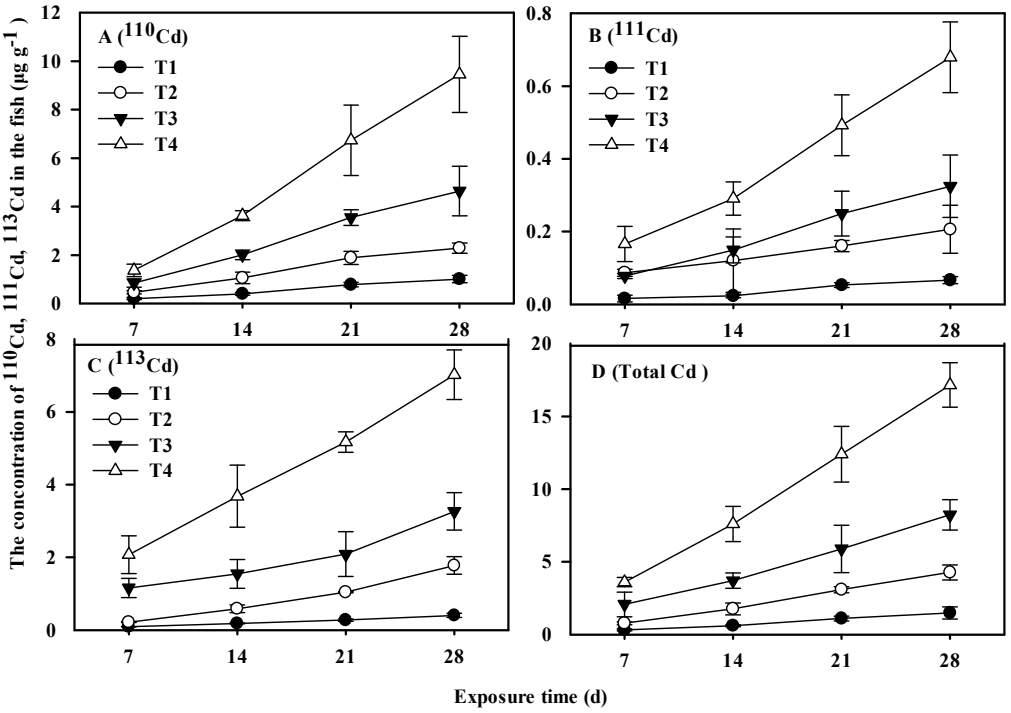

A (^{110}Cd)
B (^{111}Cd)
C (^{113}Cd)
D (Total Cd)
T1
T2
T3
T4
The concentration of ^{110}Cd, ^{111}Cd, ^{113}Cd in the fish (μg g^{-1})
Exposure time (d)

Figure 2

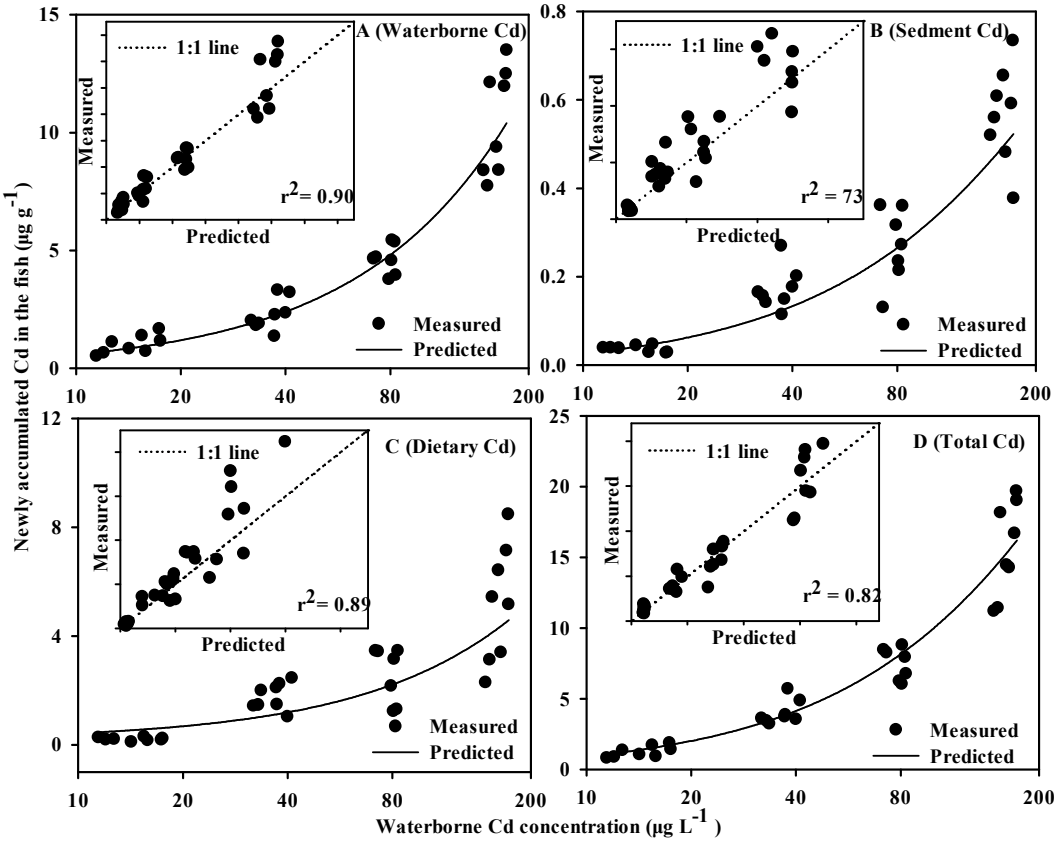
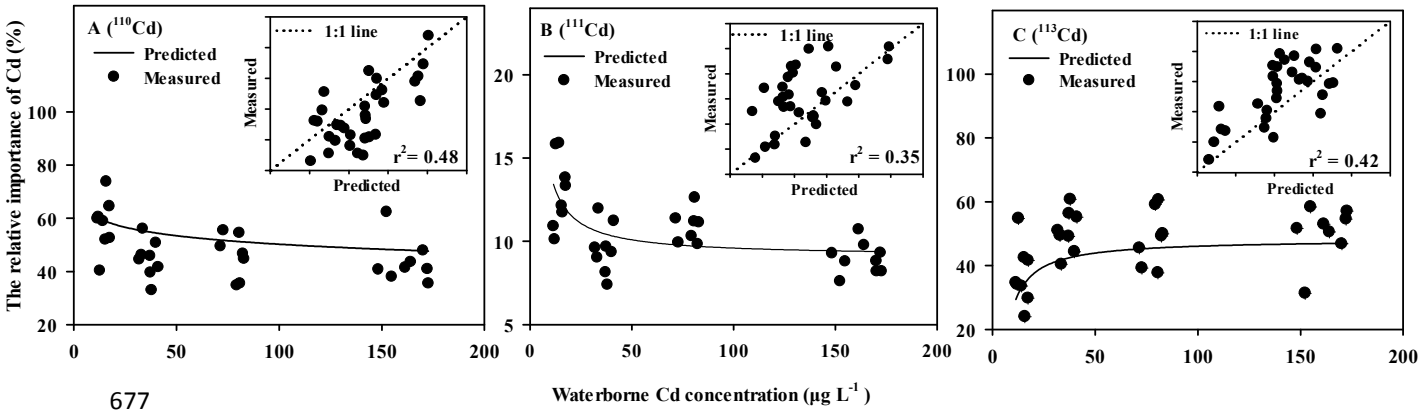

A (Waterborne Cd)
B (Sedment Cd)
C (Dietary Cd)
D (Total Cd)
1:1 line
Measured
Predicted
r2= 0.90
r2 = 73
r2= 0.89
r2 = 0.82
Newly accumulated Cd in the fish (μg g-1)
Waterborne Cd concentration (μg L-1)

Figure 3



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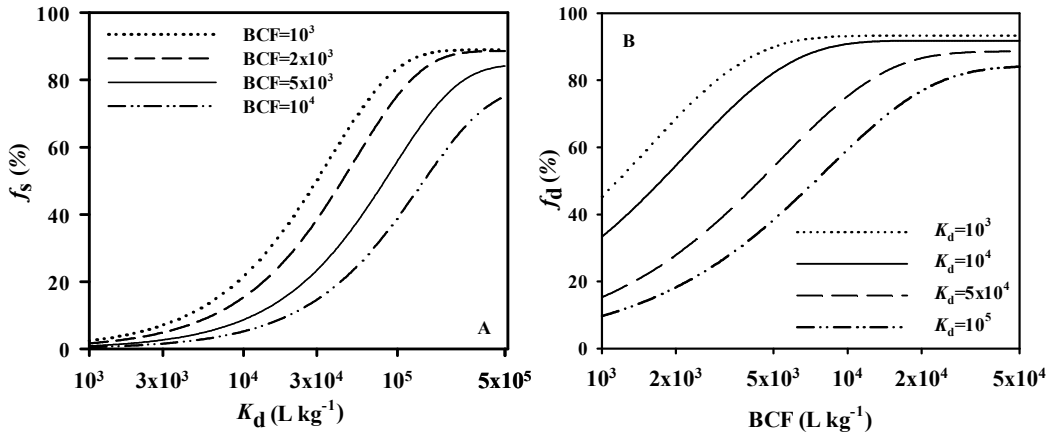
683 **Figure 4**

684



685
686
687
688
689

690 **Figure 5**

691